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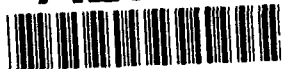
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Our data on the biology of HIV-2 suggest that this virus may have a distinct biology from that of its clost relative HIV-1. It is therefore relevant to assess these differences in populations infected with significant rates of both HIV-2 and HIV-1. Senegal is such a West African country, and the studies established there have provided important new information on the natural history and epidemiology of HIV-2. The prolonged incubation period for HIVs in general suggests that much can be learned from the evaluation of infected individuals over time. This would include: 1) the dynamics and risk factors and transmission. 2) the immune response parameters evaluated over the course of infection and their correlation with viral burden and clinical course and 3) evolution of HIV pathogenesis. Our studies conducted to date, have already indicated differences in the above virus-host interactions between HIV-2 and HIV-1. Continued comparative studies contribute to our overall understanding of HIV pathogenesis.

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INTRODUCTION

Over the past decade our appreciation of the biological diversity of human and animal retroviruses has increased dramatically. The Human Immunodeficiency Virus type 1 (HIV-1) is now well-recognized as the cause of Acquired Immunodeficiency Syndrome (AIDS) which has reached epidemic proportions in many countries worldwide. In 1985, healthy Senegalese female prostitutes were reported to have antibodies highly reactive with SIV, a recently described simian virus related to HIV-1 (1,2). This data was suggestive of a new human retrovirus more closely related to SIV and more distantly related to HIV-1. Subsequent isolation and characterization of viruses from other West Africans confirmed these findings and the virus has been termed Human Immunodeficiency Virus type 2 (HIV-2). 93-5) Early reports of HIV-2 were from AIDS patients and fears of a second AIDS pandemic were raised.

HIV-2 was given its name to indicate its close relationship to HIV-1, the prototype AIDS virus. This was based on similarities in cell tropism, major antigenic cross-reactivity and genetic properties. Despite the similarities of HIV-2 to HIV-1 from a virological standpoint, many aspects of the comparative epidemiology of these two human retroviruses are still incompletely understood. Seroepidemiologic studies have demonstrated significant rates of HIV-2 infection in West Africa, and case reports from the US and Europe indicate that the spread of HIV-2 through international travel is ongoing (5-14). It is therefore of critical importance to better understand the biology and clinical significance of HIV-2 infection and evaluate its potential as a second AIDS causing virus.

Since the discovery of HIV-2 we have been involved in a clinical prospective study of registered female prostitutes in Senegal . These studies have shown that HIV-2 may be distinct from HIV-1 in more than its geographic distribution in Africa. Our first objective for this contract has been to characterize the epidemiology and natural history of HIV-2. Three epidemiologic cohort studies of female prostitutes in Senegal have been established and evaluated for seroprevalence, seroincidence and identification of risk factors for HIV-2 and HIV-1 infection (15,16). Sub-cohorts from each site have been monitored for clinical and immunologic abnormalities to characterize the natural history of HIV-2 infection. This is a unique study population with both HIV virus types and the longest and largest prospective study of HIV infected individuals conducted in Africa.

Our second objective has been to characterize the immunologic response to HIV-2 and assess its relationship to viral load and disease progression. We have strived to identify quantitative or qualitative differences in HIV-2 antibody responses to viral structural and regulatory proteins, assess correlation with health status, time of infection and geographic origin. Virus isolation and characterization and PCR studies are in progress to assess quantitative and qualitative differences that may correlate with the natural history of HIV-2 infection.

Objective 1:
Epidemiology and Natural History of HIV-2

Our first serologic studies in Senegal in 1985 demonstrated HIV-2 antibodies in healthy female registered prostitutes from the capital city, Dakar (2,6). To further evaluate the extent of HIV-2 infection in this sexually active high risk group we surveyed other registered female prostitute populations in other parts of the country. The seroprevalence for HIV-2 varied widely between six different registered prostitute populations in various urban centers throughout Senegal (n=1920): Ziguinchor (46.2 percent), Kaolack (28.8 percent), Louga (21.4 percent), Dakar (9.8 percent), Thies (4.6 percent) and St. Louis (1.5 percent) (5,15). These data suggested that even within a self-identified high risk group, risk factors associated with HIV-2 seropositivity might vary considerably. The current study was undertaken to identify and compare the risk determinants for HIV-2 in three populations of female registered prostitutes from Dakar, Ziguinchor, and Kaolack. In addition, the presence of significant HIV-2 and HIV-1 infection in the Dakar cohort, allowed identification and comparison of risk factors for both virus types in the same self-identified risk group.

MATERIALS AND METHODS

Study population
In 1970, the government of the Republic of Senegal instituted a program for the registration of self-identified female prostitutes, legalizing their practice of providing sex for payment. This program required semi-annual evaluation and treatment if necessary for sexually transmitted diseases at clinic centers established for this purpose in Dakar (1970), Kaolack (1987), and Ziguinchor (1987). Venous blood samples were routinely taken at annual intervals to assess serologic status. These centers originally managed by social workers and nurse practitioners were joined by our study physicians in Dakar (1985), Kaolack (1987) and Ziguinchor (1987). This allowed for a more complete physical examination but also more extensive clinical assessment at scheduled and non-scheduled visits. All registered prostitutes visiting the clinics were asked to participate in the study and have their blood tested for HIV. All registered prostitutes consenting to participate, with at least one serum sampling, were included in the study, beginning in Dakar in 1985, Ziguinchor and Kaolack in 1987, with all current and subsequently registered prostitutes enrolled until December, 1990.

Questionnaire data

Basic demographic data were obtained at registration including year of birth, nationality and year of registration. A questionnaire was administered by study physicians in French and/or Wolof (a major language spoken in Senegal) upon enrollment in the study. The questionnaire allowed demographic characteristics to be verified and extended to include ethnic group. Risk determinants relating to sexual behavior included: age of first sex, years of sex with multiple partners, number of sexual partners per week and condom use. Risk behaviors related to traditional skin piercing practices were evaluated in questions relating to female excision (clitoridectomy), tattooing, and scarification. Questions regarding transfusion evaluated possible risk by this mode of transmission as well as an indication of antecedent medical problems similar to questions posed on the history of hospitalizations. History of BCG vaccination was considered as a potential marker of early health care and was also important to our assessment of cellular immunity in prospective clinical studies where skin testing for delayed type hypersensitivity reaction to this immunogen was evaluated (16). Physicians separately noted the presence of a vaccination scar as a means of assessing reliability of self-reported medical procedures.

HIV serodiagnosis

All serum samples were evaluated by immunoblot to HIV-2 (MS-U937) and HIV-1 (IIIb-Molt); no screening test was employed (2). Specimens were classified as positive if antibodies directed at *env,* ± *gag,* ± *pol* antigens were recognized. Samples with antibodies to envelope antigens only, were classified as positive if two or more *env* antigens were recognized (17). All nitrocellulose sheets impregnated with viral antigens were pre-tested with control sera to assure uniformity in serodiagnosis. Radioimmunoprecipitation analysis (RIPA) of S_{35}-cysteine labelled whole cell lysates of HIV-1 and/or HIV-2 were performed on sera with indeterminate immunoblot results, as previously described (2). Similar criteria for immunoblot serodiagnosis were employed for the RIPA assay (17).

We have previously described individuals from regions of West Africa that demonstrated antibodies seropositive for both HIV-1 and HIV-2 (6). Such "dually reactive" samples were further evaluated in an attempt to distinguish highly cross-reactive samples infected by one virus type from dual infection (17). Recombinant expressed proteins of the unique gene products of HIV-1, vpu (18) and HIV-2, vpx (19) were utilized with appropriate control sera in an immunoblot assay. Seropositivity to both vpx and vpu was consistent with at least one round of replication for both virus types, therefore compatible with dual infection. Neither of these unique gene products is uniformly immunogenic in HIV-1 or HIV-2 infected individuals.

Therefore, the lack of reactivity to either of these antigens did not affect previous immunoblot serodiagnosis (18,19).

Statistical analysis

Univariate analysis was performed on all variables from the questionnaire data to demonstrate a possible risk factor. Odds ratios (OR) were used to estimate relative risks on categorical variables. Select variables were analyzed with X^2 and Student's t-test. Analysis of risk determinants was conducted by site based on the distinct characteristics of each of the study sites and the variable prevalence for HIVs. The data for all Senegalese registered prostitutes were pooled between sites and site was considered a distinct variable in further analyses. To control for the simultaneous effect of many mutually confounding factors, a step-up logistic-regression model was constructed using GLIM (Numerical Algorithm Group, Royal Statistical Society, Oxford, England). Ten separate ethnic group categories were represented in our cohorts of women. Certain ethnic groups appeared to be associated with seropositivity and since ethnic group distribution varied significantly between study sites this variable was forced into the core model for all analyses. Subsequent models controlled for significant variables in a stepwise manner. Adjusted odds ratios (Adjusted OR) and 95 percent confidence intervals (95% CI) were calculated by using logistic regression parameter estimates.

RESULTS

Study population and prevalence of HIVs

The multi-site design of the study was implemented in order to evaluate HIV-2 infection in multiple cohorts with distinct demographic and behavioral characteristics. Each cohort showed a distinct distribution of nationalities, with the major portion being Senegalese. In Dakar, 24.8 percent (316/1275) of the registered prostitutes were non-Senegalese nationality, two-thirds of these women were Ghanian (67.4 percent; 213/316). In Ziguinchor, 39.6 percent (110/278) were non-Senegalese, the vast majority of which were from Guinea Bissau (90.9 percent; 100/110). Kaolack was the only cohort composed largely of Senegalese women (98.1 percent; 154/157).

Senegal, similar to many other African countries, is characterized by a population comprised of a large number of distinct ethnic groups, frequently speaking a distinct language. Over 20 different ethnic groups were represented in our three different cohorts of registered prostitutes. These were collapsed into ten basic groups based on known ethnologic data, as follows: 1) Wolof, 2) Serere, 3) Peuhl, 4) Toucouleur, 5) Mandinque, Bambara, Soce,

Sarankole, Dialancke and Soussou, 6) Mandjaque, Pepelle and Mancagne, 7) Diola, 8) Maure, Lebou, Balante, and Cariviano, 9) Ashanti and 10) Krobo. The latter two ethnic groupings represent major ethnic groups among Ghanians only. Preliminary analysis of ethnic group demonstrated an association to serostatus, cohort site, and various traditional skin-piercing practices. It was therefore decided *a priori* that this variable would be an important risk factor and was included in the logistic regression core model.

The distribution of other demographic characteristics and risk determinants assessed in these female prostitutes are shown on table 1. A number of potential risk determinants for HIV infection were quite prevalent in our study population. Of note, although history of hospitalization was relatively common (33.4 percent), the history of transfusion was much less frequent (10.1 percent). This would seem to indicate that transfusion was infrequently linked to hospitalization. The history of multiple injections was extremely high in this population (94.1 percent), these being multiple intramuscular injections performed in both medical and traditional healing settings. History of BCG vaccination as a marker of early health care was reported in most women (90.8 percent) . This was verified by the presence of a visible vaccination scar and viewed as a means of verifying reported health history. A high degree of concordance (94.0 percent) between the reported history of BCG vaccination and the physician's observation of a visible scar was found.

There was variation in the proportion of women with histories of cultural skin-piercing procedures. Excision which was most fi quently a non-sterile clitoridectomy performed at puberty was found in 20.2 percent of the women. Scarification of the head and neck regions, also performed at puberty, was reported in 31.4 percent of the women. Cosmetic tattooing of the face and neck, performed during adulthood, was commonly observed (57.8 percent). Reported condom use was frequent with only 12.8 percent of women reporting that they never used condoms.

All three study sites had significant HIV-2 infection over their respective study periods; Dakar 10.0 percent (128/1275), Ziguinchor 38.1 percent (106/278) and Kaolack 27.4 percent (43/157) (table 2). The HIV-1 prevalence was most significant in Dakar, 4.1 percent (52/1275). The HIV-1 prevalence in Ziguinchor was 0.4 percent (1/278) and 1.3 percent (2/157) in Kaolack. In Dakar, a total of five women had serologic profiles consistent with dual reactive status based on immunoblot and/or reactivity to HIV-1 vpu or HIV-2 vpx (17-19).

Risk determinants for HIVs by site

Select demographic characteristics and risk determinants were evaluated from the questionnaire data according to HIV status (table 1). In all three cohorts of registered prostitutes, univariate analysis showed that older prostitutes were more likely to be HIV-2 seropositive ($p < 0.05$). We assumed that the major risk factor for this group of woman was that of increased sexual activity, with age representing increased exposure to HIV via sexual transmission. We therefore created a new variable that might better quantify the length of time for sexual exposure; years of sexual activity was calculated from the age minus the age of first sexual act.

Step-up logistic-regression analysis of the Dakar cohort data revealed that a history of traditional scarification was positively associated with HIV-2 infection (Adjusted OR=1.65, 95 percent CI=1.06-2.57). In addition, increased years of sexual activity was also found to be a positive risk factor for HIV-2 infection (for each 1-year of increase the adjusted OR=1.05, 95 percent CI=1.02-1.08). Significant risk determinants in the univariate analysis that were not found to be significant in the multivariate analysis included: age ($p < 0.05$), nationality (Ghanian versus Senegalese) (Crude OR= 2.12, 95 percent CI=1.23-3.63), and history of excision (Crude OR= 0.68, 95 percent CI= 0.30-0.99). We therefore considered these risk determinants to be mutually confounding with years of sexual activity, history of scarification, or ethnic group.

Risk determinants for HIV-1 infection in the Dakar cohort differed markedly from risk determinants for HIV-2 infection. Logistic regression analysis revealed an increased risk of HIV-1 seropositivity for individuals reporting a history of hospitalization (Adjusted OR=2.12, 95 percent CI=1.11-4.03) . A shorter period of registered prostitution was also found to be associated with HIV-1 infection (Adjusted OR= 0.86, 95 percent CI= 0.79-0.95). Significant risk determinants for HIV-1 infection from the univariate analysis that were not found to be significant in the multivariate analysis included: years of sexual activity ($p < 0.05$), and being of Ghanian nationality (Crude OR= 4.34, 95 percent CI= 1.74-10.68). We therefore considered these risk determinants to be mutually confounding with history of hospitalization, years of sexual activity, or ethnic group.

In the Ziguinchor study group, 36 percent of the women were from Guinea Bissau, a country with high prevalence of HIV-2 (table 1) (6,15,20,21). In analysis of this cohort, HIV-2 seropositivity was highly associated with women of Guinea Bissau nationality (Adjusted

OR=6.27, CI=1.43-27.59), despite controlling for ethnic groups. Again, increased years of sexual activity was significantly associated with HIV-2 infection (Adjusted OR= 1.06, 95 percent CI= 1.01-1.12). Significant risk determinants in the univariate analysis that were not found to be significant in the multivariate analysis included: age (p < 0.0001), years of registered prostitution (p < 0.0037), and history of excision (Crude OR= 0.37, 95 percent CI=0.17-0.78). We therefore considered these risk determinants to be mutually confounding with nationality, years of sexual activity, or ethnic group. In contrast to the Dakar cohort, a relatively low prevalence of HIV-1 infection was found (0.7 percent). Due to the small number of HIV-1 infected women, evaluation of risk determinants for HIV-1 in this cohort was not performed.

Similar to the other two cohorts, risk determinants for HIV-2 infection in the Kaolack cohort included increased years of sexual activity (Adjusted OR= 1.11, 95 percent CI= 1.04-1.18). History of always or sometimes using condoms was found to be protective for HIV-2 infection (Adjusted OR= 0.27, 95 percent CI= 0.09-0.82). Significant risk determinants in the univariate analysis that were not found to be significant in the multivariate analysis included: age (p < 0.0001), years of registered prostitution (p = 0.006), young age of first sexual act (p = 0.05), and high number of sexual partners per week (p = 0.01). We therefore considered these risk determinants to be mutually confounding with years of sexual activity, history of condom use, or ethnic group. The prevalence of HIV-1 was low (1.3 percent), therefore, evaluation of HIV-1 risk determinants in this cohort was not performed.

Risk determinants for HIVs in Senegalese from all three sites

The final analysis for risk determinants of HIV-2 infection was conducted on Senegalese prostitutes pooled from all three cohort sites (n=1280). Women of non-Senegalese nationality were excluded since cohort specific analysis had already indicated that nationality was a significant risk factor for HIV infection. It was also hypothesized that risk determinants in non-Senegalese women would be more heterogeneous and obscure the analysis. In addition, since the preponderance of HIV-1 infected women were from the Dakar cohort, analysis was limited to evaluation of risk determinants for HIV-2 infection.

Multivariate analysis of the Senegalese prostitutes from all three sites revealed a number of risk determinants associated with HIV-2 infection. Controlling for ethnic group, women from the Ziguinchor (Adjusted OR= 4.72, 95 percent CI=2.79-7.99) and Kaolack cohorts (Adjusted OR= 3.99, 95 percent CI =2.31-6.91) were much more likely to be HIV-2 positive as

compared to women from the Dakar cohort. As had been demonstrated in the site-specific analysis, increased years of sexual activity was associated with HIV-2 seropositivity (Adjusted OR=1.07, 95 percent CI =1.05-1.10). History of excision was associated with a decreased risk of HIV-2 infection (Adjusted OR=0.47, 95 percent CI=0.27-0.85). Similarly, a previous history of BCG vaccination appeared to decrease the risk for HIV-2 seropositivity (Adjusted OR=0.53, 95 percent CI=0.29-0.95). Significant risk determinants in the univariate analysis that were not found to be significant in the multivariate analysis included: older age (p < 0.05), young age of first sexual act (p < 0.05), lower number of sexual partners per week (p < 0.05), and history of scarification (Crude OR=1.49, 95 percent CI= 1.04-2.12). We therefore considered these risk determinants to be mutually confounding with years of sexual activity, history of condom use, or ethnic group.

The use of a government based existing mechanism for registered female prostitutes allowed for a relatively uniform sampling frame in all three sites. However, participation in this study was voluntary, with 67.5 percent participation in Dakar, 83.7 percent in Ziguinchor and 72.3 percent in Kaolack. The distribution of HIV serostatus, nationality, age, and years of registered prostitution were very similar between enrolled and non-enrolled women at all sites (table 4). Therefore, study cohorts were considered representative of the registered prostitute populations at the designated sites.

DISCUSSION

Early cross-sectional surveys of registered female prostitute populations in Senegal had shown distinct differences in the prevalence of HIV-2 and HIV-1. This study was undertaken to identify and characterize risk determinants for HIVs in three sites that had previously shown distinct differences in HIV prevalence. Comparisons of enrolled versus nonenrolled groups indicated that the study cohorts were representative of the registered prostitute populations at the designated sites.

The results from this study confirmed the different rates of HIV infection in three different urban centers of Senegal. HIV-2 prevalence varied among the three study sites, with higher rates in Ziguinchor and Kaolack. Although, Dakar is the major urban center of the country, the HIV-2 prevalence was lower in this site than the others. The rates of HIV-1 infection in most West African countries, including Senegal, have been considerably lower than Central or East Africa (6, 8). In 1985, a cross-sectional survey of six urban centers in Senegal, reported the first cases of HIV-1 infection in Dakar (6).

Women of Ghanaian and Guinea Bissau nationality constituted a significant proportion of study participants in the Dakar and Ziguinchor sites, respectively. Both of these nationalities were associated with higher HIV-2 prevalence. The acquisition of either HIV infection may have occured in Senegal or in their country of origin. To date, little is known regarding the prevalence of HIV-2 in Ghana, although both HIV-2 and HIV-1 infection have been reported (20). In addition, Ghanaian prostitutes report that they migrate and work in a number of other West African countries such as Burkina Faso, Ivory Coast and Mali, all countries with significant HIV-2 prevalence. The increased HIV-2 prevalence in women from Guinea Bissau is not unexpected, in that blood donor HIV-2 prevalence has been reported as 8.3 percent (21) and prevalence in female prostitutes at 37.0 percent (15, 22). A significant proportion of HIV-1 infected women in the Dakar cohort were also of non-Senegalese nationalities, and the possibility that these women acquired their HIV-1 infection prior to immigration to Senegal must be considered.

When only women of Senegalese nationality were considered, the HIV prevalence in Ziguinchor and Kaolack was quite similar, 25.7 percent and 27.9 percent, respectively. However, the explanation for the 9.2 percent prevalence found in Senegalese women in the Dakar site, remains less apparent. Client selection may explain, in part, the differences in HIV seroprevalence rates between the different sites. Ideally, the serostatus of the prostitute's clientele would have been informative, however, identification of male clients was not possible in this study. The questionnaire data included the primary nationality of the clients of a subset of women in our study. In Dakar, 66.3 percent (350/528) of clients were Senegalese, whereas 92.5 percent (123/133) of clients were Senegalese in Ziguinchor and 97.7 percent (128/131) in Kaolack. HIV-2 positivity in the female prostitute was associated with having a Senegalese male client (p < 0.0027). It is of interest, that Senegalese clients were more frequent in the two sites with higher HIV-2 prevalence. However, it was not possible to conclude that this was the determinant responsible for the difference in HIV prevalence between the sites.

The multi-site design of the study allowed us to identify site-specific risk factors for HIV infection as well as risk determinants for the total group of women in this common risk group. In the Dakar cohort, women reporting a history of scarification were more likely to be HIV-2 seropositive, controlling for ethnic group differences. Although traditional scarification practices with non-sterile knives could conceivably be a means of HIV transmission, it would

be premature to assume that this was the mode of transmission by which these women were infected. It is possible that the history of scarification is linked to other sexual practices including client selection that might also help to explain this result.

Another site-specific risk determinant was found in the Kaolack cohort: women reporting that they never used condoms were more likely to be HIV-2 seropositive. Counselling on STD/AIDS prevention and condom distribution was performed on an individual basis in the Dakar and Ziguinchor clinics. However, in Kaolack, women would attend group sessions biweekly in addition to the individual counselling at clinic visits. It is possible that this slight difference in the intervention was responsible for differences in condom usage between sites. These data suggest that intervention programs that distribute condoms may be effective in decreasing HIV infection. However, it would also be important to evaluate other sexual practices that may associate with condom usage. Studies are currently underway to evaluate the reliability of self-reported condom use in these cohorts. Data on the incidence of other sexually transmitted agents and the identification of spermatozoa on vaginal smears may better evaluate whether women truthfully respond to questions regarding condom use. Analysis of repeat questionnaires on a select group of these women demonstrated high concordance (10 percent error variance) for most historical data but significant discordance in questions regarding sexual behavior and condom use (70-90 percent error variance, respectively) (S. Gortmaker, Harvard School of Public Health, unpublished data).

Distinct risk determinants were identified for HIV-2 and HIV-1 in the Dakar cohort where significant prevalence of both viruses was found. This provided a unique opportunity to evaluate risk determinants for both HIVs in a common high risk population. Multivariate analysis revealed that HIV-1 infected women were two times more likely to report a history of previous hospitalization. In the majority of cases, the cause of hospitalization was for gynecological procedures such as abortion or Caesarean section. There was no significant difference in seropositivity in women reporting a hospitalization within the past five years versus more than five years ago. History of hospitalization was not correlated with history of transfusion and we would therefore assume that this positive risk factor is not indicative of the mode of HIV transmission in these women. It is possible that the history of hospitalization may be indicative of antecedent medical problems which could predispose to HIV-1 infection or be a marker of early HIV-1 clinical disease. It is not known why women with a shorter duration of practicing prostitution were at higher risk for HIV-1 infection. Similar results have also been reported in HIV-1 seropositive prostitutes in Nairobi, Kenya (23). It is conceivable

that client selection and sexual practices may change during the practice of prostitution. Similarly, a woman may be more prone to active infection by other STD agents early in her prostitution career, which may increase susceptibility to HIV-1 infection. Finally, a recent increasing HIV-1 prevalence or cohort effect may be responsible for this association of HIV-1 to shorter duration of prostitution. This is substantiated by data from an incidence study conducted in this same cohort, where HIV-1 incidence increased eight-fold between 1985 and 1990 (24).

In Africa, it has been well recognized that heterosexual transmission is a major mode for HIV infection. Cross-sectional studies of sexually active risk groups such as self-identified female prostitutes have consistently demonstrated high prevalence rates of both HIVs as compared to low-risk sentinel groups (5-7, 22, 23). We have therefore assumed that the major risk factor for the women in our studies is that of increased sexual activity. It could be hypothesized that age, years of registered prostitution, number of sexual partners per week and years of sexual activity might all be variables that measure degree of potential sexual exposure to HIV. Multivariate analysis of the three cohorts as well as the pooled Senegalese cohort showed that women with more years of sexual activity were more likely to be HIV-2 seropositive. The approximate log-linear relationship of HIV-2 seropositivity with increasing years of sexual activity is consistent with the hypothesis that this virus has been in this population for at least several decades. The potential cohort effect that might produce this result, namely a recent decrease in HIV-2 prevalence, is unlikely, given the stable incidence rate of HIV-2 since 1985 (24). In distinct contrast to these results, women with fewer years of registered prostitution were more likely to be HIV-1 infected. This would seem to suggest that there is a difference in the potential risk of acquiring HIV-1 as compared to HIV-2 infection via the sexual route. Mathematical modelling of the data from our Dakar cohort of women have indicated that the infectivity of HIV-1 is approximately three times that of HIV-2 per sexual act (25).

A number of studies have indicated an association between HIV infection and other STDs, particularly those involving genital ulceration (23, 26). In sexually active risk groups, an association between HIV infection and STDs is not unexpected. However, in cross-sectional studies, it is difficult to determine whether the STD agent or agents serves as a risk factor for acquisition of HIV infection or as a co-factor in the natural history of HIV infection. Condom distribution and counselling on the prevention of STDs may be considered an intervention for both HIV and STD acquisition. The temporal effectiveness of this intervention was not

assessed in this study, and would lead to difficulties in interpretation. This study was not designed to evaluate the role of STDS in HIV infection, these complex interactions are better addressed through specifically designed longitudinal studies.

Since the discovery of HIV-2, one of the most important scientific questions asked how this related virus might differ from HIV-1 in its biological consequences. Critical to answering this question has been the comparative examination of the epidemiology and pathogenicity of HIV-2 compared to HIV-1 (27). Our study has shown that even within a self-identified sexually active risk group, the risk determinants for these two related viruses are distinctly different. Data from our ongoing prospective clinical study indicate a long incubation period for HIV-2 with a 12-fold increased relative risk for AIDS development in HIV-1 infection as compared to HIV-2 (16, 28). We have also measured HIV incidence in these women. The data indicate that HIV-2 may spread less efficiently than HIV-1 through an at-risk population (24). This is at least suggestive that the transmissibility of HIV-2 may differ from that of HIV-1. Therefore, our data suggest differences between HIV-2 and HIV-1 in risk determinants for sexually active populations, distinct incidence patterns of infection and different incubation periods to the development of AIDS. The virologic determinants and mechanisms for these apparent biological differences are still unknown. However, an understanding of how HIV-2 differs from HIV-1 at a host level is essential for interpretations of comparative virologic studies. We are hopeful that a better understanding of the epidemiology of HIV-2 and HIV-1 will also contribute to our ability to devise new and effective interventions to stem the HIV epidemic.

Natural History of HIV-2

A cohort of Senegalese prostitutes has been monitored since 1985, and an increase in the seroincidence of HIV-1 infection has been found in this population (16). This report compares the immune alterations and the rate of disease development between HIV-2 and HIV-1 in this population.

METHODS

Selection and Examination of Subjects

Cohort members were derived from women attending the "Institut d'Hygiène Sociale" (IHS) government sponsored health clinics for self-identified prostitutes in Dakar, Senegal. Since 1985, all adult women registered in the IHS clinic have been serologically screened for exposure to HIV-1 and HIV-2. The IHS provides clinical examinations, treatment of sexually

transmitted disease (STDs) and primary health care during visits which are required every 6-8 weeks for the legal registration of prostitutes. We augmented the health evaluation and services at this clinic with specially trained study physicians, educational materials, condoms, diagnostic supplies, and medications.

Throughout each year during an initial IHS clinic visit, women were assigned a unique identification number and a blood sample for HIV testing was obtained with informed consent. A demographic, behavioral and health history questionnaire, and a baseline health evaluation was performed by clinic physicians and nurse practitioners. When women returned for subsequent clinic visits, all who tested HIV seropositive were enrolled in our clinical cohort with their consent. Two seronegative comparison women were selected for each eligible seropositive woman by distribution sampling based on age (± 2 years), nationality, and years of registered prostitution (± 3 years). That is, when a seropositive woman was identified, two similar seronegative women were randomly selected from a strata of comparable women. As with the seropositive women, these women became eligible for enrollment upon subsequent clinic visits. Twice a year, lists of the eligible enrollees for the clinical cohort were generated and distributed to the IHS clinic staff who identified enrollees as they returned for visits.

At the time of enrollment, and at subsequent biannual visits, participants were examined by a study physician and a blood sample was requested. The women were also administered a more extensive questionnaire in the language of their choice focusing on sexual history, contraception, as well as antecedent medical, pregnancy and child health data. A complete physical examination was performed and included the individual's weight, vital signs, 4-8 specific data entries for each "organ system" which emphasized signs or symptoms of opportunistic infections or of neurological compromise, and entries for any other significant clinical history or signs found during the consultation. Data was also available from a gynecological exam, a cervical smear, and a general medical evaluation performed approximately every 6-8 weeks by nurse practitioners during routine IHS visits. Microbiological testing of endocervical swabs were performed as needed for diagnosis. The serological status was unknown to the on-site physician, but was available to any of the participating women on a confidential basis. All clinic patients were given condoms and information on preventing STDs. Additional diagnostic testing, medications or special clinic referrals were routinely provided as needed.

Information on women who did not return to the IHS clinic sites for a 12 month period was actively sought in the community via nurse practitioners, social workers, and physicians. Once a year a separate "lost to follow-up" data form was completed for each subject who was not seen during that year. This information included a clinical consultation at the subject's home or at another IHS clinic site, a basic follow-up visit (outside the clinic) noting health status and reasons for not returning to the clinic, or interviews with friends or family members concerning the subject's last known health status and new address, if relocated. Subjects were removed from the study if they expressed a desire to discontinue participation, if they had permanently moved out of the country, or if they had died.

Serological Evaluation

Study subjects had biannual blood samples drawn for serological determination of HIV-1 and HIV-2 exposure. All sera were tested for antibodies to each virus by immunoblot in two laboratories: the Microbiology and Virology Department at Le Dantec Hospital in Dakar and the Department of Cancer Biology at the Harvard School of Public Health in Boston. Serostatus of each serum sample was determined according to the WHO consensus recommendations of the HIV-2 Working Group{17}. Dual reactive sera to HIV-1 and HIV-2 were further confirmed by appropriate reactivity to type-specific synthetic peptides to the transmembrane glycoprotein regions of each virus type, as well as by radioimmunoprecipitation assay (RIPA). Reactivity to HTLV was determined by immunoblot reactivity to an HTLV cell lysate preparation in a cross-sectional sample of the study population.

Serological evaluation of antibodies to treponemal antigens was determined biannually by Treponemal Hemagglutination Assay (TPHA) and by Rapid Plasmin Reagent (RPR) determinations. Chlamydia antibodies were determined by Chlamydiazyme ELISA determinations (Abbott Labs). Gonococcal smears and cultures, as well as wet preps and potassium hydroxide preps for microscopic examination were performed, as needed for diagnosis.

Immunologic Studies

In addition to the biannual clinical consultations and serological determinations, additional blood samples were requested on a yearly basis to determine a subject's's complete blood count (CBC) and T-cell subsets. Ethylenediaminetetraacetic acid (EDTA) anticoagulated blood was obtained and CBCs were determined by an automated cell counter (Coulter). Manual white blood cell differentials were obtained by averaging two readings. Peripheral blood mononuclear cells (PBMCs) were prepared for subset analysis by Ficoll-Hypaque-like separation via Leukoprep cell separation tubes (Becton Dickinson). Subpopulations of lymphocytes were identified by two-color fluorescence which utilized Leu-4 (CD3) and Leu-12 (CD19) for T and B cells respectively, or Leu-3a (CD4) and Leu-2a (CD8) for T4 and T8 cells respectively. Only lymphocytes with surface (ringed) staining were counted by fluorescence microscopy. Monocytes and cells with cytoplasmic staining were excluded. The absolute subset counts were calculated by multiplying the percentage of lymphocyte subsets by the total number of lymphocytes. Inter-run variations of our technique were periodically determined by blood samples from multiple seronegative controls run simultaneously in triplicate.

Baseline tests determined that approximately 85% of our clinic population reacted to tuberculin antigen by multiple antigen skin testing (Multitest IMC, Merieux Institute, Paris). As a result, delayed type hypersensitivity skin testing to intradermal tuberculin antigen (ppd; Sclavo) was initiated on a yearly basis. Skin test reactions were read at 48 hours, and the extent of induration was recorded. Reactions were classified according to the manufacturers recommendations of reactive, limited reaction, or non-reactive.

Determination of Clinical Status

AIDS was initially defined in our population according to the World Health Organization (WHO) conference on AIDS in Bangui {30}. The major clinical signs are weight loss (at least 10% of body weight), chronic fever (intermittent or constant lasting at least one month) or chronic diarrhea (lasting at least one month). Minor clinical signs or symptoms are a persistent cough (lasting at least one month), pruritic maculopapular dermatitis, herpes zoster, oral candidiasis, chronic herpetic infection (progressive or disseminated) and generalized lymphadenopathy. In our study, AIDS was diagnosed when two major signs and at least one minor clinical sign was present. The presence of either Kaposi's sarcoma or cryptococcal meningitis alone was sufficient for an AIDS diagnosis by these recommendations.

Since HIV serology, T-cell subset determination and certain opportunistic infection diagnostic capabilities were available, our study population was also evaluated by the Centers for Disease Control (CDC) revised surveillance case definition for AIDS{31} and by the CDC Classification System for HIV Infection{32}. All of the "WHO-defined" AIDS cases also corresponded to the CDC definition of AIDS. Cases which corresponded to the CDC IV disease classification that were not defined as AIDS by either WHO or CDC criteria (CDC IVA or C-2) were evaluated separately from and combined with the AIDS cases in the analysis.

In addition to evaluating the study population by these definitions, we also gathered clinical data on other possible HIV-associated outcomes at each clinical consultation. This data was historical, physical and diagnostic in nature. The examining physician was blind to the serological status of the subject when seen as an outpatient. Other clinical states evaluated included mucocutaneous manifestations (e.g. oral ulcerations, angular chelitis, minor mycotic infections, prurigo), recurrent or severe respiratory infections, global or localized neurological findings, and an overall performance status evaluation.

Lymphadenopathy was graded by determining the maximum level of adenopathy occurring at any one visit. Generalized lymphadenopathy (LAD) was originally defined as adenopathy of greater than 1 cm in diameter in at least two extra-inguinal nodal areas, not more than one of which was cervical{31,32}. An absence of lymphadenopathy fulfilling this criteria was noted early in the study. We relaxed, therefore, the definition of generalized lymphadenopathy to adenopathy equal to or greater than 1 cm in diameter with the same nodal area criteria. The clinical outcome of LAD is defined as the presence of adenopathy with or without the presence of other significant clinical outcome.

Statistical Analysis

Statistical differences in percent seropositivity were evaluated using the Chi-square test or Fisher's exact test. Differences in continuous variables were evaluated by the t-test and Wilcoxon's test. Incidence rates, rate ratios and 95% confidence intervals were calculated for each outcome of interest. Incidence rate confidence intervals were calculated assuming a Poisson distribution. Seroincident cases that had estimated dates of seroconversion less than 18 months prior to the most recent clinical consultation were excluded as seropositive cases for this analysis. Only subjects who were initially asymptomatic according to our defined outcomes (except LAD) were included in this analysis. Person-years of observation (PYO) for each subject was determined by calculating the time from the initial basic health evaluation and serostatus determination until the most recent clinical consultation or removal from the study. We are assuming under this determination of PYO that a significant HIV-associated outcome, such as CDC IV-type disease, did not occur yet remain undetected between the initial clinic health evaluation with serostatus determination and the enrollment visit with our study physician. This assumption was validated by reviewing the subjects' clinic records and by obtaining a full medical history upon enrollment.

RESULTS

Description of Study Population

Of the 636 women eligible to enter the study after ongoing serostatus determination and stratification, 353 returned for enrollment into the clinical cohort. This analysis considered clinical follow-up data from February 1985 until April 1991. Except for nationality, the demographic information available on the eligible registered prostitutes who did not return for enrollment was quite similar to the information obtained on those who were enrolled. Senegalese women, however, were somewhat more likely to be enrolled in the clinical cohort than women from Ghana (or from other countries). Some of the demographic features

of the enrolled study population are shown in Table 5. Subjects were generally in their mid-thirties (range 22-60), were registered prostitutes for 4 to 8 years, and were sexually active since 16-17 years of age. HIV-1 seropositive women were slightly younger than the other groups, and were registered prostitutes for a shorter period of time. Due to the distribution sampling for the comparison seronegatives, the HIV-1 and HIV-2 seropositive women were similar to the seronegative women in terms of age, years of registered prostitution, and years of sexual activity. Senegalese women represented 69% and 78% of the HIV-2 seropositive and seronegative women, respectively. Women from Ghana comprised approximately 50% of the HIV-1 seropositives in the clinical cohort and 18% of the total seronegative subjects. Additional descriptive and historical information is provided in Table 5.

As noted, the present analysis excluded seropositives who were known to have recently seroconverted. The population included 7 HIV-1 seropositive women and 12 HIV-2 seropositive women whose time of seroconversion was estimated from previous serum samplings prior to presentation for enrollment into the clinical cohort. Four subjects showing dual seropositivity to both HIV-1 and HIV-2 were also enrolled in the clinical cohort, but were excluded from most analyses due to their small number.

Prospective Observation Data

The mean time interval from the initial to the most recent evaluation were similar for HIV-2 seropositive and seronegative women (40 months and 44 months, respectively), while the mean time interval for observation of HIV-1 seropositives was less (35 months). This difference was partially due to an increased seroprevalence and seroincidence of HIV-1 in the overall clinic population in the more recent years of the study{33}.

As was done at the beginning of each calendar year, the clinic staff actively investigated the status of women who had not attended the clinic for 12 months or more, as determined each January 1st. A total of 90 women were investigated. Follow-up activities resulted in 21 women immediately returning for a clinic visit and 8 women who were seen, but not examined in the clinic. Twenty five of the investigated women had either moved out of the country (n=23) or Dakar (n=2) in apparent good health. The clinic staff was unable to obtain any pertinent information on the remaining 36 women. Thus by the 1991 analysis period, there was follow-up information on 90% of the 353 original enrollees, with current vital status data on 82.7% and with 80.5% of the enrollees continuing to visit our study physicians in the clinic within the last 12 months.

We analyzed factors associated with missing a clinic visit within the last 12 months. HIV-1 and HIV-2 seropositive women were actually less likely to miss than seronegative women. After stratification according to nationality, this association was not statistically significant. Finally, neither HIV-1 nor HIV-2 infection was associated with missing the last visit in a logistic regression model controlling for potential confounders (age, nationality, presence of anergy to ppd and CD4+ T lymphocyte count at baseline). Also, none of the potential confounders were associated with missing a recent clinic visit. (data not shown).

Initial Immunological Measurements

We compared certain immunological measurements in HIV-1 or HIV-2 seropositive individuals to seronegatives in a cross sectional manner. As summarized in Tables 6a and 6b, women seropositive to either HIV-1 or HIV-2 showed significantly lower median T4 lymphocyte counts and T4/T8 ratios (p < .001). For the HIV-1 seropositive group, median T8 lymphocyte counts were greater than for seronegatives (p=.036). None of the 4 individuals with dual HIV-1/ HIV-2 seropositivity had T4/T8 ratios less than 0.5.

Despite similar trends in T-cell alterations, a direct comparison of the two seropositive groups showed milder changes among the HIV-2 group, and a greater degree of immunosuppression in the HIV-1 group. Compared to 31.8% (7/22) of the HIV-1 group with a T4/T8 ratio ≤0.5, only 6.9% (5/72) of the HIV-2 group had an initial T4/T8 ratio ≤0.5. As compared to the HIV-2 group, HIV-1 seropositives had lower T4/T8 ratios (p=.001) and a trend towards lower absolute T4 counts (p = .065). Total lymphocyte counts were similar between the HIV-2 and HIV-1 seropositive groups.

Response to delayed hypersensitivity testing to tuberculin antigen (ppd skin test) was assessed at the initial ppd skin testing. Among the 284 women with initial skin test results, 31 of 193 (16.2 percent) HIV-seronegative women were anergic to ppd testing. The prevalence of anergy to ppd was significantly greater among HIV-infected women. Among the HIV-1 infected group, 13 of the 22 women tested (59%) were anergic whereas 21 of the 69 HIV-2 infected women tested (30%) were anergic. Table 7 presents the relative prevalence (RP) for anergy according to HIV serostatus compared to seronegative women. Both the HIV-1 seropositive group and the HIV-2 seropositive group showed significant associations with anergy. The relative prevalence for anergy for the HIV-1 group compared to the seronegative group was 5.61 (95% Confidence Interval (CI) 2.57, 12.28) For the HIV-2 group compared to the seronegative group, the relative prevalence was 1.77 (CI 1.17, 2.67). Comparing the

HIV-1 group to the HIV-2 group revealed that HIV-1 infected women were significantly more likely to be anergic than HIV-2 infected women with a relative prevalence of 2.89 (CI 1.16, 5.06).

Most women were also tested for HTLV infection. When analyzed in relation to the hematologic and immunologic parameters tested, HTLV-seropositive women were more than twice as likely to be anergic to ppd (RP=2.15, CI 1.6, 3.7) than HTLV-seronegative women. Infection with HTLV, therefore, was assessed as an effect modifier and a confounder to the association of HIV-1 or HIV-2 infection with anergy to ppd. Only one woman who was HIV-1 seropositive was also HTLV-seropositive. Nine women were HIV-2 seropositive and HTLV-seropositive. There was no significant effect and only a small amount of confounding by HTLV infection. The Mantel-Haenzel adjusted RP for anergy was 6.26 (CI 2.82, 14.78) for HIV-1 infection and 1.17 (CI 1.10, 2.66) for HIV-2 infection.

Clinical Outcomes
The incidence rates (IR) and the 95% confidence intervals (CI) for selected major clinical outcomes by serostatus are presented in Table 8. All incidence rates are calculated per 100 PYO. After a total of 888 PYO among the seronegative women, we noted one clinical case that would be classified as CDC IV disease, not AIDS-defining, had the case been seropositive. Two deaths have occured in this seronegative group to date. Among the 88 HIV-2 infected women we observed one AIDS case after 292 PYO, which also represents the one death in this group (IR=0.3; CI 0.1,1.9). In addition, 7 women developed CDC IV disease conditions, which were not CDC (or WHO) AIDS defining (IR=2.4; CI 1.2 - 500). The total CDC IV disease cases observed, therefore, were 8 for the HIV-2 seropositives (IR=2.7; CI 1.2, 5.4). Among the 24 HIV-1 infected women, we saw 3 AIDS cases after 69 PYO, which accounted for the 3 deaths in this group (IR=4.4; CI 1.5, 12.8). Five other women had manifested CDC IV disease conditions which were not AIDS defining (IR=7.3; CI 3.1, 17.0). The total number of CDC IV disease cases in the HIV-1 seropositives seen was 8 (IR=11.6; CI 5.9, 22.9). Among the 4 HIV-1/HIV-2 dual seropositive women enrolled who were seen for a total of 7 PYO, there was one AIDS case (IR=14.3; CI 2.5, 80.9). This woman is presently alive and under treatment (data not shown).

LAD was noted at enrollment in 1 seronegative woman, 4 HIV-2 seropositive women and in no HIV-1 seropositive women. During the study, the incidence of LAD, whether or not coincident with other disease outcomes was seen in 5 of 236 seronegative women, (IR=0.56; CI 0.24, 1.32), in 9 of 24 HIV-1 seropositive women (IR=13.04; CI 6.86, 24.79), and in 13 of 84

HIV-2 seropositive women (IR=4.61; CI 2.69, 7.89). The rate ratio (RR) of HIV-1: HIV-2 incidence rates for LAD was 2.83 (CI 1.21, 6.62).

Most interesting were the rate ratios of incidence rates for the major clinical outcomes by serostatus. This data is shown in Table 9. The rate ratios were consistently and significantly elevated for the incidence of all major HIV-associated conditions when the HIV-1 seropositive group was compared to the HIV-2 seropositive group. HIV-1 infected women had 12.7 times the rate of development of AIDS or death as HIV-2 infected women (CI 1.3, 122.1). HIV-1 infected women also had 3 times the rate of development of CDC IV disease which was not AIDS defining (CI 1.0, 9.5). By combining these two categories, the rate of CDC IV disease development was 4.2 times more likely in HIV-1 infected women than in HIV-2 infected women (CI 1.6, 11.3)

DISCUSSION

Most reports surveying the clinical manifestations of individuals infected with HIV-2 have previously been case-series{34-39} or cross-sectional in nature{40-42}. These types of studies are important in describing the epidemiological and clinical status of HIV-2 infected individuals. When controls are utilized, cross-sectional studies may also demonstrate disease association. In one study in West Africa, researchers attemped to obtain follow-up data on initially hospitalized AIDS cases seropositive for either HIV-1 or HIV-2{40}. However, when hospital-based surveys are used to identify index cases or subsequent cohorts of infected individuals, the apparent pathological effects of exposure to HIV-2 (or HIV-1) may be amplified by ths type of case selection.

In general, disease association has been assessed by previous studies, but only prospective outpatient studies can assess the rate of disease development with HIV-2. Our present investigation is the first report of a prospective, controlled survey of initially asymptomatic HIV-2 seropositive individuals. Of note in the present survey is the possible comparison to the clinical outcome of HIV-1 infection in the same setting and study population.

As shown in this population, the incidence rates for AIDS is distinct for HIV-2 and HIV-1. By examining the rate ratios for AIDS between HIV-1 and HIV-2, we see that the likelihood of developing AIDS with HIV-2 is significantly less than with HIV-1 infection. Although we cannot predict the global disease causing potential of HIV-2 from one study, we feel confident in our results particularly since the rates of disease progression in the HIV-1 seropositive

women are what might be predicted from other studies of the rate of AIDS development in asymptomatic HIV-1 seroprevalent cohorts (Table 10,ref. 50).

Numerous studies have looked at the rate of progression of AIDS and HIV-related conditions in HIV-1 cohorts in North America and Europe (reviewed in ref.{43-46}). The rate of progression in African populations has been studied less frequently and until this report, the average observation period was not longer than 2 years. One study in Nairobi suggests that the rate of HIV-1 progression in their study population may be higher than expected{47}. The range of rates of progression and the rarity of natural history studies in an African setting, make the HIV-1 seropositive subjects in the same cohort in this study more pertinent when attempting to examine the rate of disease development of HIV-2 infection. One might argue that the rate differences seen between HIV-1 and HIV-2 in this cohort are due to more recent exposure to HIV-2 versus HIV-1 in this cohort. In other words, HIV-1 seropositives have been infected earlier than the HIV-2 group and are at a later stage of infection or disease. The available data , however, supports the opposite conclusion. It appears that HIV-1 seropositive individuals in this cohort would be more likely to have been infected later than HIV-2 seropositives for the following reasons.

First, HIV-2 was serologically verified to have been in the region since the mid-1960's{48,49}. HIV-1 was first reported in surveys in Senegal in 1985{1}. Second, the seroconversion rate for new HIV-2 infections in this same population of prostitutes in Dakar is quite constant{33}. The seroconversion rate for new HIV-1 infections, however, was low the first 2 years of observation and has risen markedly since, regardless of nationality. Finally, the age-specific seroprevalences for HIV-2 in this population and in others has been shown to increase with older age groups. This situation is different than HIV-1 age-specific seroprevalences and more indicative of an infectious agent that has been in the population for some time.

Ideally, one would like to know the exact time of infection for each person in a prospective study. Seroprevalent cohorts can be useful, however, if certain assumptions are noted. With the use of person years of observation, we have made the assumption that the hazard of disease development with HIV-1 or HIV-2 is fairly constant each year after excluding the first 1-2 years of seropositivity. The rate of AIDS development in the non-African setting may actually rise somewhat in HIV-1 infection after 60 months of seropositivity{43}, but in general the rate of AIDS development with HIV-1 infection is somewhat constant for each year of seropositivity after the first 1-2 years of infection. The validity of the assumption that HIV-2 has a similar disease hazard model for time of seropositivity is unknown at present.

This report further documents the intermediate immune alterations seen when HIV-2 infection is compared to HIV-1 infection, and to seronegatives in an outpatient population. These intermediate alterations include both the results of T-cell subsetting and of delayed-type hypersensitivity skin testing. We found that our seronegative control group had elevated T4 lymphocyte subset counts when compared to ranges reported for non-African populations. We were reassured that this may be primarily a population, rather than a technique-related phenomenon when other surveys of seronegative women in Africa produced similar ranges for these values{47}. Realizing that caution should be excercised when using T4/T8 ratios to evaluate HIV immune alterations, the use of the T4/T8 ratio was also used for evaluation to compensate for the higher baseline hematologic values seen in this population. The further characterization of immune parameters in HIV-2 seropostives in comparison to controls should now take place in a prospective fashion.

Studies concerning the natural history of infections or disease are difficult to achieve in any setting. We feel that our study will continue to document the distinct natural histories of HIV-2 versus HIV-1 infection. Our study has attempted to minimize the participants lost to follow-up, which is critical to any prospective survey. Those individuals that have been lost to follow-up were not more likely to be HIV seropositive, nor to have clinical or immune changes indicative of early HIV disease. The high rate of follow-up was attained by a dedicated and energetic network of nurses, social workers and physicians.

OBJECTIVE 2:
Characterization of the Immune response and Viral Carriage of HIV-2.

The understanding of the differences in the epidemiology and natural history of HIV-2 infection compared to HIV-1, leads to obvious questions regarding differences in the immune response to these viruses. The basic premise would be that virus-host immune response interactions would be responsible for differences in longer clinical incubation periods, transmission and overall pathogenesis. Studies conducted under the previous contract, indicated similarlity in gross quantitative and qualitative humoral immune response to HIV-2 antigens. These studies have looked at other accessory gene products of HIV-2 as well as epitopes of the envelope antigen.

Our main objective in these studies was to evaluate the utility of various serologic markers for use in distinquishing HIV-1 from HIV-2 infection. In addition, we wished to assess differences in humoral immune response to certain viral antigens that might provide useful data for disease prognosis. These included recombinant expressed env peptides from HIV-1 and HIV-2, and recombinant-expressed vpx and vpu proteins the unique gene products of HIV-2 and HIV-1, respectively.

Serum samples were obtained from West African individuals previously serodiagnosed by whole viral lysate immunoblots to HIV-1 (IIIb) and multiple HIV-2 isolates (MS-U937, NIH-Z and ST). Semi-purified recombinant-expressed HIV-1 (566) and HIV-2 (966) env proteins, homologous with the N-terminal region of gp41 (51) and gp35 (52), have been described. Recombinant-expressed vpu (HIV-1) (18) and vpx (HIV-2) (19) have been described. All recombinant expressed proteins were analyzed by immunoblot.

Results
Reactivity of HIV-2 positive samples on recombinant expressed vpx was much less, with 8.4% (19/227) reactivity and no reactivity on vpu (0/227), 65 HIV negatives were vpx negative. Dual reactive sera showed comparable rates to vpx and vpu and singly infected individuals.

Antibody Reactivity to Recombinant HIV-1 *vpu* and HIV-2 *vpx*

	HIV-1 *vpu*	HIV-2 *vpx*
HIV-1 Seropositive Central & West Africa and Mexico	34/81 (42%)	0/81 (0%)
HIV-2 Seropositive	0/227 (0%)	19/227 (8.3%)
HIV Dual Reactive West Africa	44/112 (39%)	11/112 (10%)
HIV Negative Central & West Africa and Mexico	0/65 (0%)	0/65 (0%)

The HIV-2 seropositive samples detected the HIV-2 recombinant env peptide (996) 100%(40/40) of the time with 0% (0/40) cross-reactivity to the HIV-1 peptide (566) (see table below). HIV-1 seropositive samples from 4 diverse geographic origins demonstrated 100% reactivity (77/77) to the HIV-1 specific peptide (566) with substantial cross-reactivity 57% (44/77) to 996. Dual-reactive sera detected both recombinant peptides 100% (37/37). None of the HIV negative sera reacted nonspecifically to these envelope peptides.

Antibody Reactivity to Recombinant *env* peptides (566/996)

	HIV-1 566	HIV-2 996
HIV-1 Seropositive Central & West Africa USA and Mexico	77/77 (100%)	44/77 (57%)
HIV-2 Seropositive Senegal	0/28 (0%)	28/28 (100%)
Guinea Bissau	0/12 (0%)	12/12 (100%)
HIV Dual Reactive	37/37 (100%)	37/37 (100%)
HIV Negative Central & West Africa USA and Mexico	0/17 (0%)	0/17 (0%)

The 556 HIV-1 env peptide was found to be type-specific, whereas the 996 HIV-2 peptide demonstrated significant cross-reactivity. Vpx reactivity was not found to be useful in confirming HIV-2 infection in all of the cases, its use as a prognostic marker is under evaluation.

In addition to our assessment of the biology of HIV viruses in Senegal, we have concurrentl) evaluated the presence of HTLV viruses as well. Sera from 1021 individuals and 8 periphera blood lymphocytes (PBL) DNA were obtained from female prostitutes from our cohort study ir Dakar and Ziguinchor, Senegal. Immunoblot was performed using HUT-102 cell lysate as antigen on all sera, RIPA was used for confirmation. Sera from 103 individuals (10%) met the

criteria of HTLV positivity (reactivity to 2 viral bands) and 16 (1.6%) were indeterminate (reactive with 1 viral band). All indeterminate samples were further evaluated by RIPA .

All blot positive sera and indeterminate sera were analyzed on recombinant-expressed HTLV-I (B-I) and HTLV-II (II-B) envelope proteins (Chen et al.) by immunoblot. Among the 103 HTLV positive individuals 71 (69%) had antibodies against B-I, suggesting an HTLV-I infection, and 5 against II-B only. One sample showed strong reactivity suggestive of HTLV-2 infection, PBL DNA was not available. None of the blot indeterminate samples showed reactivity to the HTLV recombinant env proteins.

In collaboration with Bruce Walker at Mass General Hospital we have been studying the generation the cytotoxic lymphocytes directed to HIV-2. Target cells for these assays consisted of autologous EBV lymphoblasts infected with recombinant vaccina virus expressed the HIV-2 ROD and ST env genes, the SIV251 env gene, SIV gag gene, HIV-2 RT gene and a control vaccina virus. Effector cells consisted of: 1)Fresh unstimulated PBMCs; 2) CD8+ enriched lymphocytes, which had been expanded in vitro with interleukin2; and 3) PBMCs cloned at limiting dilution using a CD3-specific monoclonal antibody as a stimulus to T cell proliferation.

HIV-2 env-specific CTL activity was detected in 4/6 seropositive subjects. In 3/4, no env-specific activity was detected in fresh PBMCs or expanded CD8+ cells, although clones were obtained from these individuals which recognized SIV251env. In 1/4, ST-specific activity was detected in the expanded CD8+ lymphocytes, although no clone could be isolated from this subject. In 1/6 subjects, there was marked gag-specific CTL activity, which could be detected using target cells expressing the SIV gag protein. Two epitopes within gag were mapped using synthetic viral peptides. The HLA-restriction of these epitopes is under investigation. RT-specific activity was not detected in any subject, and in 2/6 subjects there was no detectable CTL against any gene using these methodologies.

CONCLUSIONS

In addition to prospective data for modeling of the HIV-2 and HIV-1 epidemics in West Africa, follow-up studies such as this one may provide prognostic information for the thousands of HIV-2 seropositive persons. Also, further investigations into basic differences in biology or pathogenesis of HIV-1 versus HIV-2 should be encouraged by these findings. One should not minimize, however, the potential consequences of HIV-2 infection by this data. Only after further studies are completed concerning the transmission rates of HIV-2, the natural history

of the diseases associated with HIV-2 and the follow-up of seroincident cohorts, can the full impact of this immunodeficiency virus be appreciated.

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TABLE 1. Demographic characteristics of registered female prostitutes by Human Immunodeficiency Virus type 1 (HIV-1) and type 2 (HIV-2) in Dakar, Ziguinchor and Kaolack, Senegal, 1985-1990.

Population Characteristics	Dakar			Ziguinchor			Kaolack		
	No.*	% HIV-2 seropositive†	% HIV-1 seropositive	No.	% HIV-2 seropositive	% HIV-1 seropositive	No.	% HIV-2 seropositive	% HIV-1 seropositive
Study population	1275	10.0	4.1	278	38.1	0.4	157	27.4	1.3
Nationality									
Senegal	959	9.2	2.4	168	25.7	0.6	154	27.9	1.3
Ghana	213	13.2	9.8	5	0.0	0.0	0	0.0	0.0
Guinea Bissau	12	50.0	0.0	100	60.0	0.0	2	(1/2)	0.0
Other Nationalities	91	6.6	8.8	5	(1/5)‡	0.0	1	0.0	0.0
Age (years)									
20-29	350	7.4	4.6	74	23.0	0.0	32	12.5	0.0
30-39	655	9.3	4.1	141	38.3	0.0	85	22.3	1.2
40-49	225	12.0	4.0	45	51.1	2.2	29	44.8	3.4
50-59	40	27.5	0.0	14	64.3	0.0	10	(7/10)	0.0
60-69	5	(3/5)	0.0	2	(1/2)	0.0	1	(1/1)	0.0
Years of registered prostitution									
1-9	902	9.9	5.2	278	38.1	0.4	106	23.6	1.9
10-19	351	10.0	1.4	0	0.0	0.0	51	37.2	0.0
20-29	22	18.2	0.0	0	0.0	0.0	0	0.0	0.0
Years of sexual activity									
0-9	122	4.9	8.2	26	19.2	0.0	5	(1/5)	0.0
10-19	553	8.3	4.5	95	29.5	0.0	57	15.8	0.0
20-29	386	9.8	3.4	62	41.9	1.6	41	31.7	4.9
30-39	70	14.3	0.0	19	57.9	0.0	17	70.6	0.0
40-49	13	61.5	0.0	3	(2/3)	0.0	5	(4/5)	0.0

* No., number of prostitutes with characteristic, this serves as denominator of the percentage for each stratum. Dual reactives removed.

†%, percentage of prostitutes with characteristic and seropositive by stratum.

‡ ( ), raw proportion for small numbers.

TABLE 2. Profile of behavior determinants in registered female prostitutes by Human Immunodeficiency Virus type 1 (HIV-1) and/or type 2 (HIV-2) in Dakar, Ziguinchor and Kaolack, Senegal, 1985-1990.

Population characteristics	Dakar			Ziguinchor		Kaolack	
	No.*	% HIV-2 seropositive†	% HIV-1 seropositive	No.	% HIV-2 seropositive	No.	% HIV-2 seropositive
Total study population	1275	10.0	4.1	278	38.1	157	27.4
With a history of hospitalization	329	7.0	3.3	103	36.9	41	21.9
With a history of transfusion	88	9.1	2.3	32	28.1	22	22.7
With a history of multiple injections	646	11.9	3.4	172	36.7	144	28.5
With a history of scarification	328	12.2	3.3	65	30.8	54	31.5
With a history of excision	197	7.1	1.5	56	21.4	23	39.1
With a history of tattoo	593	8.4	2.0	89	32.6	123	28.4
With a history of BCG vaccination	848	8.1	2.8	203	34.5	147	26.5
Condom use							
always	307	9.1	2.6	23	26.1	14	0.0
sometimes	501	7.6	2.8	110	34.5	90	22.2
never	107	5.6	2.8	7	(3/7)	40	47.5

* No., number of prostitutes with this response, this serves as denominator of the percentage for each stratum. Dual reactives removed.

†%, percentage of prostitutes with response and seropositive by stratum.

‡ ( ), raw proportion for small numbers.

TABLE 3. Risk determinants associated with HIVs in Dakar, Ziguinchor, Kaolack, and Senegalese prostitutes from all three sites, 1985-1990.

Study Site	HIV Type	Risk Determinant*	Odds Ratio†	95% CI‡	Risk Association
Dakar	HIV-2	Years of sexual activity	1.64	1.26-2.14	Increased
		History of scarification	1.65	1.06-2.57	Increased
Dakar	HIV-1	Years of registered prostitution	0.23	0.09-0.59	Decreased
		History of hospitalization	2.12	1.11-4.03	Increased
Ziguinchor	HIV-2	Years of sexual activity	1.80	1.08-2.98	Increased
		Guinea Bissau nationality	6.27	1.43-27.59	Increased
Kaolack	HIV-2	Years of sexual activity	2.73	1.49-5.19	Increased
		History of condom use	0.27	0.08-0.82	Decreased
All Senegalese prostitutes (3 sites)	HIV-2	Site (Ziguinchor)	3.99	2.30-6.91	Increased
		Site (Kaolack)	4.72	2.79-7.99	Increased
		Years of sexual activity	2.00	1.57-2.55	Increased
		History of excision	0.47	0.27-0.85	Decreased
		History of BCG vaccination	0.53	0.29-0.95	Decreased

* For risk determinants expressed in years, statistics are based on 10 year difference. Senegalese and Dakar are used as reference for nationality and site, respectively.

† Odds ratios were calculated by multiple logistic analysis. Odds ratio for a single variable, adjusting for ethnic group in the core model.

‡ CI, confidence interval.

TABLE 4. Distribution of nationality, age, years of registered prostitution and serostatus by enrolment in the study in Dakar, Ziguinchor, and Kaolack, Senegal, 1985-1990.

Population Characteristics	Dakar				Ziguinchor				Kaolack			
	Enrolled		Not Enrolled		Enrolled		Not Enrolled		Enrolled		Not Enrolled	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total Population	1284	67.2	627	32.8	278	83.7	54	16.3	157	72.4	100	27.6
Nationality												
Senegalese	963	75.0	389	63.0	168	60.4	14	51.9	154	98.1	97	97.0
Ghanaian	218	17.0	194	31.4	5	1.8	0	0.0	0	0.0	0	0.0
Guinea Bissau	12	0.9	3	0.5	100	36.0	11	40.7	2	1.3	0	0.0
Other	91	7.1	31	5.0	5	1.8	2	7.4	1	0.6	3	3.0
Age (years)												
20-29	352	27.4	153	25.4	74	26.8	10	37.0	32	20.4	24	28.9
30-39	660	51.4	314	52.2	141	51.1	12	44.4	85	54.1	44	53.0
40-49	227	17.7	106	17.6	45	16.4	4	14.8	29	18.5	10	12.0
50-59	40	3.1	22	3.7	14	5.1	0	0.0	10	6.4	5	6.0
60-69	5	0.4	7	1.2	1	0.4	1	3.7	1	0.6	0	0.0
70-79	0	0.0	0	0.0	1	0.4	0	0.0	0	0.0	0	0.0
Years of registered prostitution												
1-9	910	70.9	439	70.1	278	100.0	50	100.0	106	67.5	80	80.0
10-19	352	27.4	175	28.0	0	0.0	0	0.0	51	32.5	20	20.0
20-29	22	1.7	12	1.9	0	0.0	0	0.0	0	0.0	0	0.0
Serostatus												
HIV-1	48	3.7	9	1.6	1	0.4	0	0.0	2	1.3	0	0.0
HIV-2	124	9.7	44	7.9	106	38.1	12	22.2	43	27.4	20	20.0
HIV-2/HIV-1	9	0.7	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
Negative	1103	85.9	452	81.6	171	61.5	28	51.9	112	71.3	35	35.0
Not determined *	0	0.0	48	8.7	0	0.0	0	0.0	0	0.0	45	45.0

* Serologic analysis not performed or results considered indeterminant.

Table 5. Demographics, Socioeconomic Data and Baseline History of Registered Prostitutes in the Dakar Clinical Cohort

	HIV Serostatus		
	Negative n=237	HIV-2 n=88	HIV-1 n=24
Age in years, mean ± SD	35.8 ± 7.4	37.2 ± 8.6	33.8 ± 6.0
Range	24-60	23-60	22-43
Nationality, n (%)			
Senegalese	184 (77.7)	61 (69.3)	9 (37.5)
Ghanaian	43 (18.1)	(22.7)	12 (50.0)
Other*	10 (4.2)	7 (8.0)	3 (12.5)
Years of registered prostitution, mean ± SD	7.9 ± 4.9	7.6 ± 4.9	4.0 ± 3.9
Total years of sexual activity, mean ± SD	19.7 ± 7.8	21.9 ± 9.8	16.3 ± 6.0
Sexual partners per week, mean ± SD	8.5 ± 9.9	9.9 ± 10.8	12.8 ± 12.0
Oral contraceptive use, n (%)	28/172**(16.4)	3/59 (5.1)	7/20 (35.0)
Charge per client, n (%)			
≤ 500 CFA***	9/134 (6.7)	10/48 (20.8)	3/17 (17.7)
501 - 5000 CFA	67/134 (50.0)	26/48 (54.2)	10/17 (58.8)
> 5000 CFA	58/134 (43.3)	12/48 (25.0)	4/17 (23.5)
Subsequent condom use, n (%)			
always	66/198 (33.3)	27/65 (41.5)	10/22 (45.5)
sometimes	115/198 (58.1)	32/65 (49.2)	10/22 (45.5)
never	17/198 (8.6)	6/65 (9.2)	2/22 (9.1)
History of formal education, n (%)	79/165 (47.9)	19/58 (32.8)	14/17 (82.4)
Religion, n (%)			
Muslim	138/166 (83.0)	40/56 (70.2)	8/17 (47.0)
Christian	28/166 (17.0)	16/56 (28.1)	9/17 (52.9)
History of ethnic scarification, n (%)	83 (35.0)	42/87 (48.3)	12 (50.0)
History of clitorectomy, n (%)	39 (16.5)	11/87 (12.6)	3 (12.5)
History of tatoo, n (%)	146 (61.6)	47/87 (54.0)	7 (29.2)
BCG vaccination scar, n (%)	177 (74.7)	60/87 (69.0)	19 (79.2)
History of transfusion, n (%)	21/236 (8.9)	7/87 (8.0)	2 (8.3)
History of past hospitalization for infectious disease†, n (%)	8/228 (3.5)	1 (1.1)	2 (8.0)

* Includes individuals from Ivory Coast, Guinea, Guinea-Bissau, Nigeria and Sierra Leone
** Denominator indicates proportion of serostatus group represented
*** 250 CFA ≈ 1 U.S. dollar
† Acute diarrhea, miscellaneous infectious diseases, tuberculosis, and/or other pneumopathies

TABLE 6a.   HEMATOLOGIC VALUES AT INITIAL DETERMINATION FOR 353 DAKAR CLINICAL COHORT MEMBERS
1989-1991

	SERONEGATIVE n=202* median (interquartile range)	HIV-2 SEROPOSITIVE n=72 median (interquartile range)	HIV-1 SEROPOSITIVE n=22 median (interquartile range)	HIV-1 vs HIV-2 p**	HIV-2 vs SERONEG. p	HIV-1 vs SERONEG. SERONEG. p
WBC x 10^6 cells/ml	6600 (5500-7700)	6400 (5300-7800)	6050 (4800-7650)	.511	.260	.400
Lymphocytes x 10^6 cells/ml	3079 (2443-3784)	2548 (2100-3205)	2673 (1865-3808)	.911	.100	.001
T4 cell count x 10^6 cells/ml	1673 (1352-2071)	1216 (851-1552)	769 (568-1540)	.065	<.001	<.001
T8 cell count x 10^6 cells/ml	946 (728-1254)	1028 (776-1355)	1249 (729-1831)	.205	.036	.172
T4/T8 ratio	1.70 (1.38-2.23)	1.20 (0.87-1.56)	0.77 (0.44-1.10)	.002	<.001	<.001

TABLE 6b.   DISTRIBUTION OF T4/T8 T-CELL RATIOS BY SEROSTATUS

T4/T8 RATIO	SERONEGATIVE		HIV-2 SEROPOSITIVE		HIV-1 SEROPOSITIVE		Comparison of Distributions of T4/T8 Ratios		
							HIV-1 vs HIV-2 p***	HIV-2 vs SERONEG p	HIV-1 vs SERONEG p
	N	%	N	%	N	%			
≤0.5	1	0.5	5	6.9	7	31.8			
>0.5 to 1.0	9	4.5	19	26.4	9	40.9	.001	<.001	<.001
>1.0	192	95.0	48	66.7	6	27.3			
	202	100.0	72	100.0	22	100.0			

* Values include 202 of 237 seronegatives, 72 of 88 HIV-2 seropositives and 22 of 24 HIV-1 seropositives.
** Wilcoxon rank-sum test

Table 7.   **ASSOCIATION OF ANERGY TO SKIN TESTING AND HIV SEROSTATUS**

	HIV-1 to HIV-2	HIV-1 to Seronegative	HIV-2 to Seronegative
Relative Prevalence (95% CI)	2.42 (1.16, 5.06)	5.61 (2.57, 12.28)	1.77 (1.17, 2.67)
Adjusted for HTLV Infection, Mantel Haenzel Relative Prevalence	3.00 (1.38, 6.50)	6.46 (2.82, 14.78)	1.71 (1.10, 2.66)

note: n=193 seronegative, 22 HIV-1 seropositive and 69 HIV-2 seropositive subjects.

Table 8. INCIDENCE RATES (IR) OF HIV-RELATED DISEASE ACCORDING TO HIV SEROSTATUS

PER 100 PERSON YEARS OBSERVATION (PYO) IN DAKAR PROSTITUTES

	Negative n=237 PYO=888			**HIV-2** n=88 PYO=292			**HIV-1** n=24 PYO=69		
	n	IR	[95%CI]	n	IR	[95%CI]	n	IR	[95%CI]
AIDS	0	-	-	1	0.3	[0.1 - 2.0]	3	4.3	[0.9 - 12.7]
CDC IV Disease- not AIDS-defining*	1	0.1	[.02 - 0.6]	7	2.4	[1.01 - 4.9]	5	7.2	[2.3 - 16.9]
CDC IV Disease-all	1	0.1	[.01 - 0.6]	8	2.7	[1.18 - 5.4]	8	11.6	[5.0 - 22.8]
All Deaths	2	0.2	[0.1 - 0.8]	1	0.3	[0.1 - 2.0]	3	4.3	[0.9 - 12.7]

*Conditions included in CDC IVA and C-2, that are not AIDS-defining conditions.

Table 9. COMPARING INCIDENCE RATES OF

CLINICAL OUTCOMES BY RATE RATIOS (RR)

ACCORDING TO SEROSTATUS

	HIV-1 to HIV-2		HIV-2 to Seronegative		HIV-1 to Seronegative	
	RR	[95% CI]	RR	[95% CI]	RR	[95% CI]
AIDS	12.7	[1.3 - 122]	----		----	
CDC IV Disease - not AIDS-defining	3.0	[1.0 - 9.5]	21.3	[2.6 - 73]	64.4	[7.5 - 551]
CDC IV Disease-all	4.2	[1.6 - 11.3]	----		----	
All Deaths	12.7	[1.3 - 122]	1.5	[0.1 - 16.8]	19.3	[3.2 - 115]

Table 10. COMPARAISON OF THE DAKAR COHORT RESULTS WITH APPROXIMATED AIDS INCIDENCE RATES OF PREVALENT COHORTS

VIRUS INFECTION STUDIED	LOCATIONS	RATE / 100 PYO
HIV-2	Dakar[*]	0.3
HIV-1	Dakar[+]	4.3
HIV-1	USA[**]	3 – 6
HIV-1	Kinshasa[***]	7.4
HIV-1	Nairobi[****]	12.5

* present report
** represents an approximate range of AIDS incidence rates reported from numerous studies (43-45).
*** represents AIDS incidence rate from most recent report (50).
**** represents estimate of AIDS incidence from data presented (46).